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TECHNIQUES FOR ESTIMATING THE AGE AND GROWTH OF MOLLUSCS: CEPHALOPODA

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ABSTRACT Global abundances and commercial fisheries of cephalopods have increased over recent decades, creating a need for effective fishery management. This management is often focused on the ability to ascertain the age structure of key populations. There are several main techniques for age and growth rate determination in cephalopods. Because of biological differences between species groups, not all techniques are applicable for every species. This review outlines the use of five main age-registering structures (statoliths, gladii, beaks, stylets, and eye lenses) along with one chemical aging technique (lipofuscin) and their application to cephalopod species groups.

INTRODUCTION

The Cephalopoda represents the most evolutionarily advanced class of the mollusca, comprising over 800 species (Wood 2017). The dominant group Coleoidea (with one extant subdivision—Neocolioidea) includes species with an internal shell, such as squid (Teuthida), bobtail squid (Sepiolida), cuttlefish (Sepiida), octopus (Octopoda), and Vampyromorpha. Cephalopod fisheries are widespread and their relatively short life spans and high stock fluctuations make these dynamic fisheries a challenge to manage. Global cephalopod abundance has increased (Doubleday et al. 2016) with their landings quadrupling (from 1 to 4.3 million tonnes [FAO 2009]) in recent decades (Arkhipkin et al. 2015).

In contrast to the Bivalvia (and to an extent Gastropoda) in lieu of an external shell, there is a diverse range of structures and techniques which can be used for age and growth rate estimations. Because of the high value and scale of many cephalopod fisheries, some of the aging techniques described in this review are routinely used as standard procedure for management and monitoring (e.g., Arkhipkin 1993). Others are still in their infancy, but hold great promise for future research.

STATOLITHS

Statoliths are solid calcareous concretions located in the statocysts (equilibrium organs) of coleoid cephalopods including squid, cuttlefish, sepiolids, and octopods (Clarke 1966) (Fig. 1). Statoliths of octopods contain randomly arranged statoconia without any visible increments in their microstructure (Clarke 1978). On the contrary, statoliths of squid, sepiids, and sepiolids accrete calcium carbonate from the statocyst endolymph with constant periodicity (Jackson 1994, Arkhipkin 2005). The rate of accretion depends on the time of the day, resulting in the appearance of translucent and wider layers of aragonite crystals interspaced by opaque and narrow layers of organic matrix (Fig. 2). Similarly to growth increments in otoliths of larval and juvenile fish (Panella 1971), it has been assumed that growth increments within squid statoliths are formed on a daily basis (Hurley et al. 1979, Lipinski 1980, Rosenberg et al. 1980). Later, daily periodicity in squid statoliths has been validated using chemical marking methods, whereby the number of growth increments formed after marking can be compared with the number of days elapsed because of marking (Dawe et al. 1985, Hurley et al. 1985, Lipinski 1986, Nakamura & Sakurai 1991). Growth increments start to form either in the beginning of the larval phase (ommastrephid squid) or during late embryogenesis (loliginid squid). Hence, their total number from hatching mark to the edge of the statolith represents the age of an individual squid in days. Age estimations based on statolith aging should be treated with caution (Rodhouse 1991) until the growth increments are validated for a given species (Lipinski 2001).

Resolution of growth increments within the statolith microstructure varies in different parts of statoliths and in different populations and species of coleoid cephalopods. In some large squid like *Onykia robusta* (Verrill, 1876) (Onychoteuthidae), growth increments are hard to discern in the peripheral zone of adult statoliths. Statoliths of some squid (*Sthenoteuthis oualaniensis*) and cuttlefish (*Sepia officinalis*) have reduced organic matrix that prevents the formation of easily resolved growth increments. It is recommended to use other hard structures such as the gladius or beak to estimate the total age of these animals (Arkhipkin & Shcherbich 2012).

Various procedures were suggested to make the statolith aging techniques less time and labor consuming (Dawe & Natsukari 1991). Since then, statoliths have been used as a routine method to estimate age and growth in squid, but these

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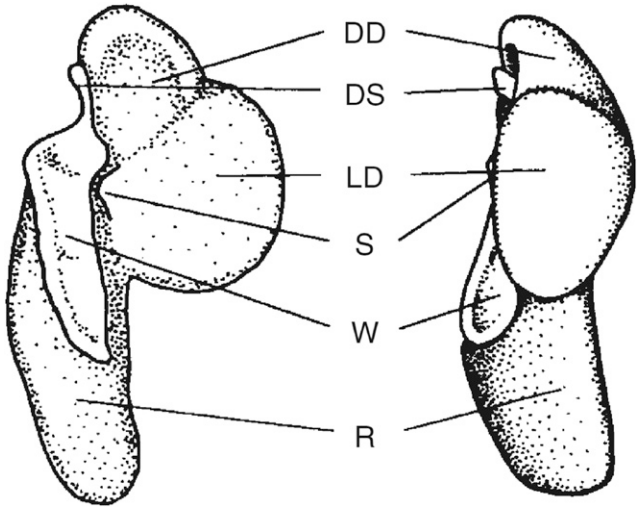

Figure 1. Diagrammatic representation of statoliths from the squid *Berryteuthis magister*. Anterior and lateral views are presented on the left and right, respectively. Dorsal dome (DD), lateral dome (LD), dorsal spur (DS), statolith body (SB), spur (S), wing (W), and rostrum (R). Reproduced with permission from Arkhipkin, 2005, from CSIRO publishing.

studies were mainly based on a small sample size (<100 specimens) and often used only a part of squid ontogeny, such as juvenile or subadult periods (Jackson 1994, 2004, Arkhipkin 2004).

Arkhipkin and Shcherbich (2012) overviewed various statolith preparation and processing techniques and suggest how statolith aging can be improved. Statoliths of both larval and juvenile squid, as well as small squid, have a translucent surface and do not need any grinding to examine their microstructure. Statoliths of adult animals need to be ground on both sides until the starting point of the statolith growth (focus) and the statolith edge are fully exposed. Various fine abrasive media are used for grinding, such as waterproof sandpaper (600–1,200 grit) or alumina powder. As the shape of statoliths varies among different families and genera (Clarke 1978), choice of grinding plane is important to reveal (if possible) the whole sequence from the statolith nucleus to the edge in one section. Statoliths grow three-dimensionally with uneven growth rates in different parts. The final goal is to prepare a section of the statolith part containing the widest growth increments that are easy to discern. In most loliginids and some oegopsids such as *Berryteuthis*, *Chtenopteryx*, and *Ancystrocheirus*, the rostral part of the statolith should be ground, whereas in most oegopsids the lateral and/or dorsal domes should be sectioned. Lateral dome has also been used for aging *Sepia* species (Perales-Raya et al. 1994, Bettencourt & Guerra 2001). The resolution of growth increments is improved by embedding the sections into a mounting medium (such as Canada balsam) that also masks the scratches on the ground surface. The best resolution of growth increments can be achieved if they are parallel to the transmitted light of the microscope. As the statolith is a three-dimensional structure, the direction of growth of its various parts changes during ontogeny, making it impossible to choose a grinding plane where growth increments of the whole section are parallel to the transmitted light. It is, therefore, recommended to use the least curved structure. The thickness of the statolith section is also important. If it is too thick (>100 μm), parts of growth increments located outside the focal plane would create obscuring shadows. A statolith section of ~50 μm thickness should contain growth increments parallel to the focal plane improving their resolution. Grinding sections that are too thin (<30 μm) deteriorate the resolution of growth increments as there is not enough optical contrast between translucent and opaque parts of the growth increment. In most cases, the ground sections of statoliths are observed under the transmitted light of a compound microscope under 400–800× and count by a reader either directly through eyepieces of the microscope, or on the computer screen using the camera mounted onto the microscope.

Despite time- and labor-consuming preparation and reading of statoliths, these hard structures are most common to use in estimations of age and growth of most species of squid, cuttlefish, and sepiolids. Short life cycles of modern coleoids require aging of large amounts of statoliths in relatively short period of time to be able to reveal the growth of various intrapopulation cohorts. Development of advanced computer software that will enable the counting of growth increments from live images of the microscope camera is still needed to reveal growth rates and population dynamics and to assist with stock assessment of coleoid cephalopods around the world.

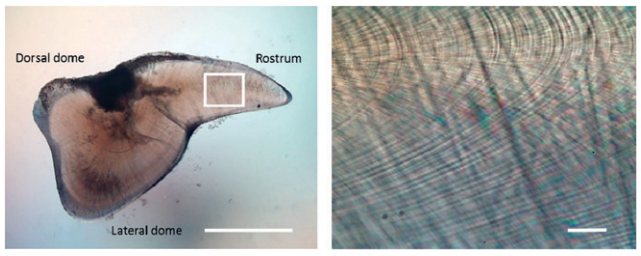

Figure 2. Prepared section of the statolith of *Doryteuthis gahi* ground both sides (on the left) and daily growth increments within the rostrum (on the right). Scale bars: 500 μm on the left and 20 μm on the right.

GLADII

According to the classical definition of Adolf Naef (Naef 1921), the term gladius could be applied to any kind of the vestigial shell of modern and extinct teuthid molluscs. Modern definition limits this term only to the chitinous skeletal structures located in the dorsal mantle of vampyromorphs, oegopsids, myopsids, and sepiolids (Fuchs et al. 2007, Arkhipkin et al. 2012). At the same time, vestigial shells of Cirrate octopuses are traditionally named gladii as well (Aldred et al. 1983, Bizikov 2008). In the framework of this section, only the term gladius will be used in reference to decalcified internal shells of modern squid (suborders Oegopsida and Myopsida) and bobtail squid (order Sepiolida) to avoid confusion.

According to Bizikov (1987), typical modern gladius consists of three structural layers: inner, intermediate, and outer shell layers (Fig. 3A). Gladii of bobtail squid and several squid families (Chtenopterygidae, Thysanoteuthidae, Mastiogoteuthidae, Chiroteuthidae, and Cranchiidae) consist only of two, or even one (Bathyteuthidae), layers (Bizikov 2008). Each of these layers grows periodically during the animal's life, forming a complex composite structure (Bizikov 1991). Shape and visibility of increments forming each layer are different; in

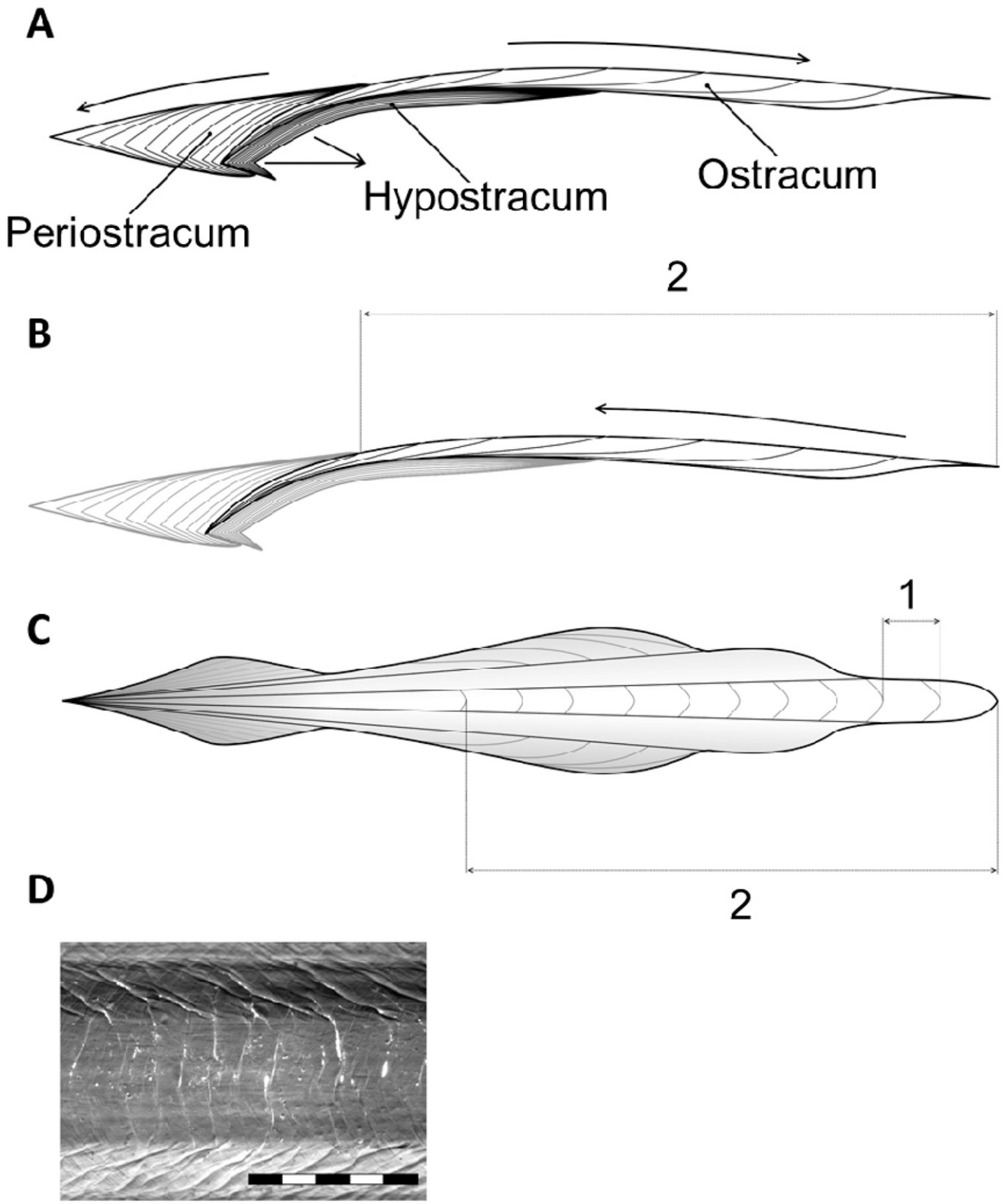

Figure 3. General structure of cephalopod gladii. Arrows indicate the growth direction of each layer. (A) Increment counting on the intermediate layer of the gladius. (B) Lateral view, thick arrow indicates the direction of counting. (C) Dorsal view. (D) Growth increments on the dorsal surface of rachis of *Berryteuthis magister* (Berry, 1913). 1—Single increment, 2—zone of counting. Scale bar, 5 mm.

particular, increments of the inner layer and rostrum have a form of consecutive bands or concentric rings, respectively, and are readable only on sectioned samples. At the same time, increments in the intermediate layer appear as pronounced striae on the dorsal surface of the gladius (La Roe 1971). Thus, increments on all three layers of the gladius could be used for age estimation. The level of development of the whole gladius and each of the layers varies significantly among species (Liu & Chen 2010), so it is easier to use the largest part of the gladius with the widest and best resolved growth increments (Bizikov 1991). As it was noted earlier, in most cases this layer is the intermediate layer.

Gladius processing for age estimation can be divided into four stages: extraction, preservation, sample preparation, and reading. The first and second stages are identical for increment counting on every layer. Gladii are extracted from fresh or formalin-preserved specimens, through the incision on the inner or outer (depending on mantle thickness of particular species) dorsal mantle surface (Bizikov 2008). For long-term preservation and storage, gladii are rinsed in fresh soapy water and preserved in a 4% solution of formalin (Bizikov 1991, Song et al. 2012). Gladii selected for reading are rinsed from the fixative in fresh water and measured (length) to the nearest 1 mm (Bizikov 1991, Perez & O'Dor 2000, Perez et al. 2006, Schroeder & Perez 2013). Further processing depends on the layer and method chosen.

Increment reading on the intermediate layer requires little sample preparation, and in most species, counting is possible on the intact gladius. Optionally, the gladius can be marked at 1 cm intervals from the posterior to the anterior end (Bizikov 1991, Perez et al. 2006) or dried at room temperature (Song et al. 2012). Studies have showed that drying can cause deformation of the gladius and complicates counting, whereas marking could be performed using image processing software. Growth increments are observed on the central part (rachis) of the dorsal surface of the gladius (Fig. 3D) under a zoom microscope at 20–70× using a combination of reflected and transmitted light (Bizikov 1991, Perez & O'Dor 2000). Increments are counted from the anterior to the posterior tip of the rachis (Fig. 3B). It should be noted that in the approximately first third (posterior most) of the rachis, increments are unreadable (Fig. 3C), so it is impossible to estimate directly the total age of the animal using this method (Perez & O'Dor 2000, Perez et al. 2006, Song et al. 2012). Intermediate layer increment counts can be corrected using other age-registering structures. In this case, age at gladius length at which increments become faint is added to the increment count (Perez et al. 2006). This approach allows the total age of the animal to be calculated. On the other hand, there is a linear relationship between gladius increment widths and mantle growth, which allows individual growth to be reconstructed using the size of the mantle (Bizikov 1991).

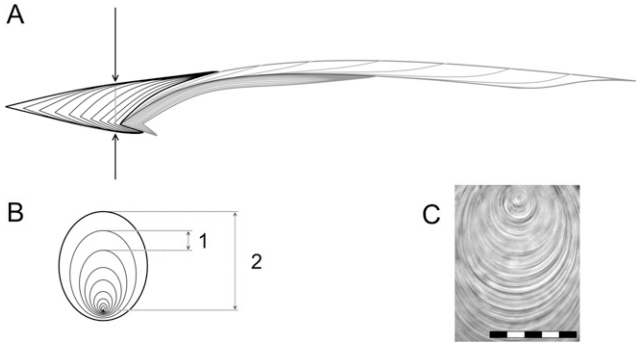

Figure 4. Increment counting on the outer layer of the gladius. (A) Lateral view, arrows indicate cutting position. (B) Cut section of the outer layer. (C) Growth increments on the outer layer of an *Onykia robusta* (Verrill, 1876) gladius. 1—Single increment, 2—zone of counting. Scale bar, 500 μm.

Counting increments on the inner and outer layers requires additional sample preparation. Primarily, the gladius should be sliced with a sharp razor or microtome knife into a number of short (20–50 mm, depending on the thickness of gladius) fragments which will be used for the preparation of sections (Bizikov 1991). Individual fragments are clamped between two blocks of Styrofoam of equal or slightly bigger size. It is necessary to make slots of appropriate size in the blocks to avoid overcompression of the gladius. Then the fragment clamped in the foam block is cut (Figs. 4A and 5A); in that, the blade moves not strictly transversely to the section but at a small angle (Bizikov 1991, Bizikov 2008). Obtained sections are placed on a slide in a drop of water, glycerol, or melting glycerol-gelatinous gel and covered with another slide (Bizikov 1991). Water and glycerol are better for immediate use, whereas samples preserved in the glycerol gelatinous gel can be stored for further examination. Counting of increments is performed under a compound microscope at 150× to 400×, using transmitted light (Fig. 4B). Both gladius layers processed this way have some benefits and limitations for age estimation (Figs. 4C and 5C). Sections of the outer layer provide a source for precise estimation of animal total age, but counts should be checked using other recording structures (statoliths or beaks) because in some species, increments are formed presumably on a subdaily basis (Arkhipkin & Bizikov 1991). Increments on the inner layer are formed daily (Perez et al. 2006), but because of the structure of the layer (during the process of laminae formation, new ones are slightly shifted anteriorly), it is difficult to estimate the total age of the animal using this method (La Roe 1971, Bizikov & Arkhipkin 1997).

To sum up, each layer of the cephalopod gladius could be used in age and growth studies. The most appropriate use of the inner layer is the test of the counts obtained using other structures (i.e., amount of increments on the statolith or beak cannot be less than those on the inner layer of the gladius), but it cannot be used in age determination and estimation of growth rates. The outer layer could be used in estimation of age and is especially good in cases when there are time limitations on sample processing (Arkhipkin & Bizikov 1991, Bizikov & Arkhipkin 1997). But before it is applied on unstudied species, periodicity of increment formation should be evaluated. Finally, the intermediate layer is the most promising gladius layer for aging studies. This layer allows direct comparison with the length of the mantle, which could also be used as an express method for age estimation (Arkhipkin & Bizikov 1991).

STYLETS

As previously discussed, statolith and gladius analyses are unsuitable for octopus species. Stylet increment analysis was the first direct aging method developed for octopus (Reis & Fernandes 2002, Doubleday et al. 2006). It involves the

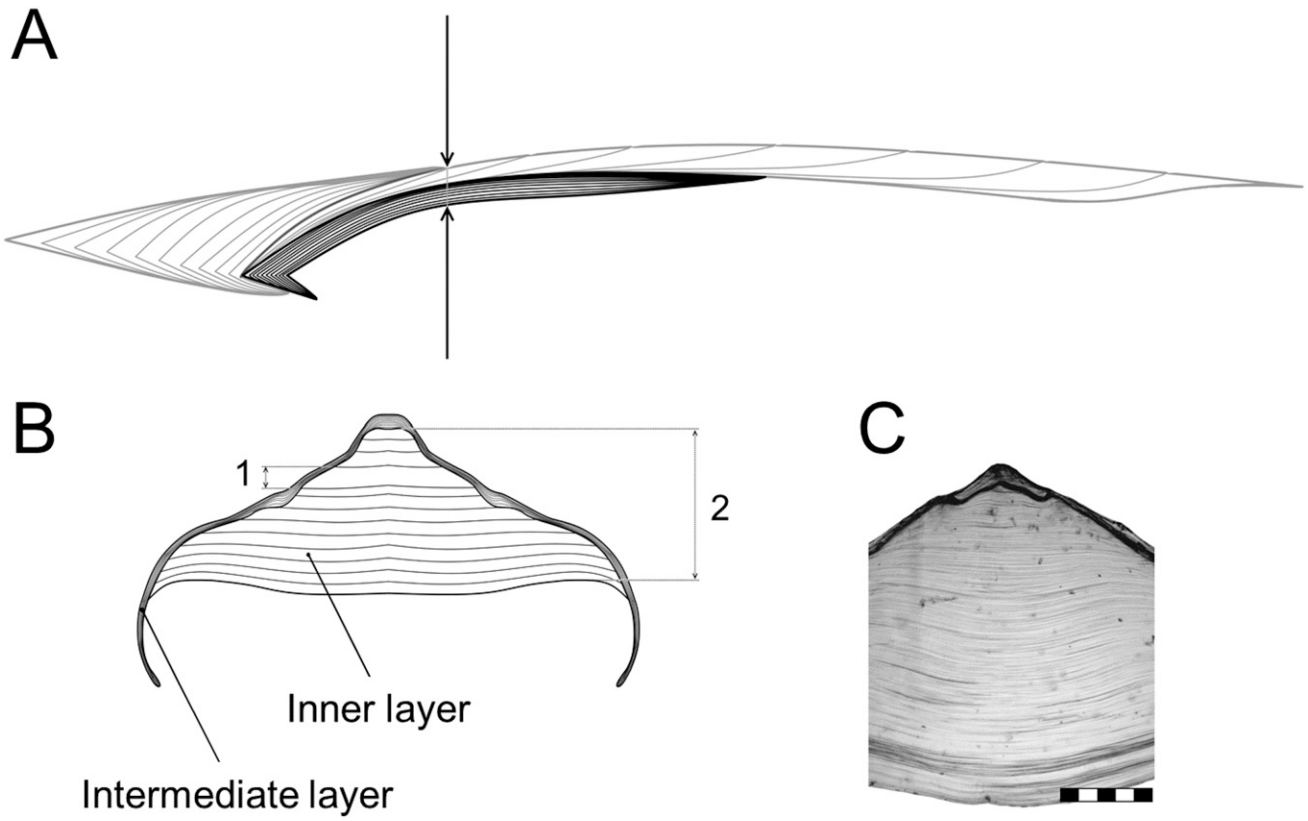

Figure 5. Increment counting on the inner layer of the gladius. (A) Lateral view, arrows indicate cutting position. (B) Cut section of the inner layer. (C) Growth increments on the inner layer of a *Berryteuthis magister* (Berry, 1913) gladius. 1—Single increment, 2—zone of counting. Scale bar, 1 mm.

enumeration of daily growth increments within stylets, hard rod-like structures (i.e., vestigial shells) embedded within the dorsolateral side of the octopus mantle (Bizikov 2004) (Figs. 6 and 7). Stylets are prepared by taking thin transverse sections, which are then viewed using light microscopy. Stylet increment analysis has been successfully applied to a range of octopus, including both holobenthic and merobenthic species (Table 1). Stylet increment analysis is not suitable for all species (one identified to date) because of variation in stylet structure and increment readability (e.g., *Macroctopus maorum*, Doubleday et al. 2011) and alternative aging methods may need to be applied (e.g., beaks, Perales-Raya et al. 2010, and lipofuscin, Doubleday & Semmens 2011). Nonetheless, preparatory methods have evolved, improving increment readability and permanency of stylet thin sections (Barratt & Allcock 2010, Rodríguez-Domínguez et al. 2013), and may be particularly useful for difficult species. Stylets are an effective aging tool and can be advantageous over other hard aging structures (see

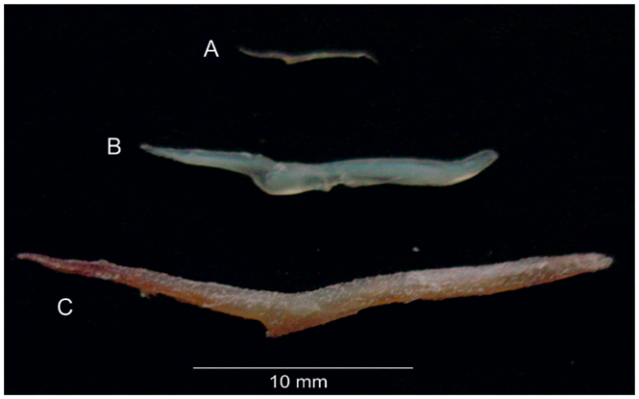

Figure 6. The stylets of *Octopus pallidus*, removed from (A) a 3-mo-old laboratory-raised specimen, (B) an 8-mo-old laboratory-raised specimen, and (C) an adult specimen collected from the wild.

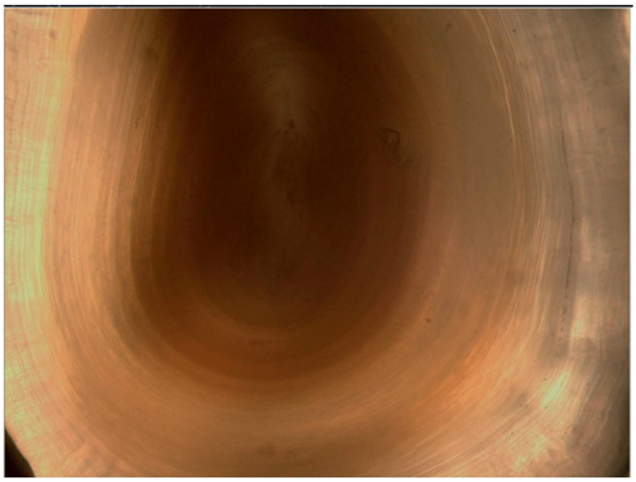

Figure 7. Transverse section of *Octopus pallidus* stylet (400×) showing concentric growth increments.

TABLE 1.

Octopus species that have been aged using stylet increment analysis.

Species	Validated	Study
Holobenthic		
Octopus pallidus	Yes	Doubleday et al. (2006), Leporati et al. (2008)
Octopus maya	Yes	Rodríguez-Domínguez et al. (2013)
Bathypolypus sponsalis	No	Barratt and Allcock (2010)
Megaleledone setebos	No	Barratt and Allcock (2010)
Merobenthic		
Octopus cyanea	No	Herwig et al. (2012)
Octopus vulgaris	Yes	Hermosilla et al. (2010), Lourenço et al. (2015)
Eledone cirrhosa	No	Regueira et al. (2015)
Octopus tetricus	No	Ramos et al. (2014)
O. (cf) *tetricus*	No	Leporati et al. (2015)

Holobenthic species refers to octopuses with large benthic hatchlings and merobenthic octopus refers to octopuses with small planktonic paralarvae. Validated = increment periodicity and first posthatch increment validated.

Rodríguez-Domínguez et al. 2013). To obtain accurate absolute age from increment counts, it is necessary to validate increment periodicity and identify the first posthatch increment (Campana 2001). Daily increment periodicity was first validated in the stylets of *Octopus pallidus* (Hoyle, 1885) (Doubleday et al. 2006) and subsequently in *Octopus vulgaris* (Hermosilla et al. 2010) and *Octopus maya* (Rodríguez-Domínguez et al. 2013) using known-age animals and chemical marking methods. The first posthatch increment has also been identified in these species by examining the stylet structure in day-old hatchlings or paralarvae and matching increment counts to known-age individuals (Doubleday et al. 2006, Rodríguez-Domínguez et al. 2013, Lourenço et al. 2015). Life history strategies vary among octopuses (i.e., holobenthic versus merobenthic species) and it will be important to continue to validate both increment periodicity and position of the first posthatch increment in more species.

More recently, studies have also shown that stylet weight is an effective proxy of octopus age and, thus, a cost- and time-effective alternative to daily increment enumeration (Rodríguez-Domínguez et al. 2013, Leporati & Hart 2015). Validating the relationship between stylet weight and the number of increments within the stylet (i.e., age) is a crucial step to ensure the accuracy of the method.

BEAKS

Beaks (or jaws) are present in all extant cephalopod species (Mangold & Bidder 1989); therefore, improvements in their preparation technique for aging purposes are potentially applicable to any cephalopod species, many of which are commercially exploited and have a high economic value. These structures are basically composed of a chitin–protein complex (Hunt & Nixon 1981) and are secreted by a single layer of tall columnar cells known as beccublasts that are responsible for their growth (Dilly & Nixon 1976, Uyeno & Kier 2005). A gradient of increasing stiffness, pigmentation, and protein, together with a decreasing content of water and chitin, has been reported from the posterior to the anterior region of the beak (Miserez et al. 2008, Tan et al. 2015). Growth process takes place from the posterior border of the beak, where the most recent chitinized and hydrated material is deposited.

Growth increments in cephalopod beaks were reported for the first time in the 1960s for the squid *Onykia ingens* (Clarke 1965) using the inner surface of lateral walls (Fig. 8A, B), although the first attempt to estimate age in these structures was carried out in the common octopus *Octopus vulgaris* in the 1990s (Perales-Raya & Hernández-González 1998). They developed a technique sectioning the rostrum region (Fig. 8C), where a sequence of regular increments was laid down in the beak microstructure, suggesting a constant daily deposition. Several studies have been conducted in octopuses using these techniques and suggesting daily deposition (Hernández-López et al. 2001, Perales-Raya et al. 2010, Cuccu et al. 2013, Perales-Raya et al. 2014a, among others). Daily formation of beak increments was documented for reared octopus paralarvae up to 26 days old (Hernández-López et al. 2001) and in certain adult size classes (Canali et al. 2011, Rodríguez-Domínguez et al. 2013, Villegas-Bárcenas et al. 2014) and validated across the full ontogenetic range (Perales-Raya et al. 2014b). The latter study also confirmed life-event recording in the beak sections as darker marks related to environmental and/or biological stress (e.g., capture). Subsequently beak increments have been used for age estimation in squid species in which daily deposition was confirmed by comparing with statolith-determined ages (e.g.,

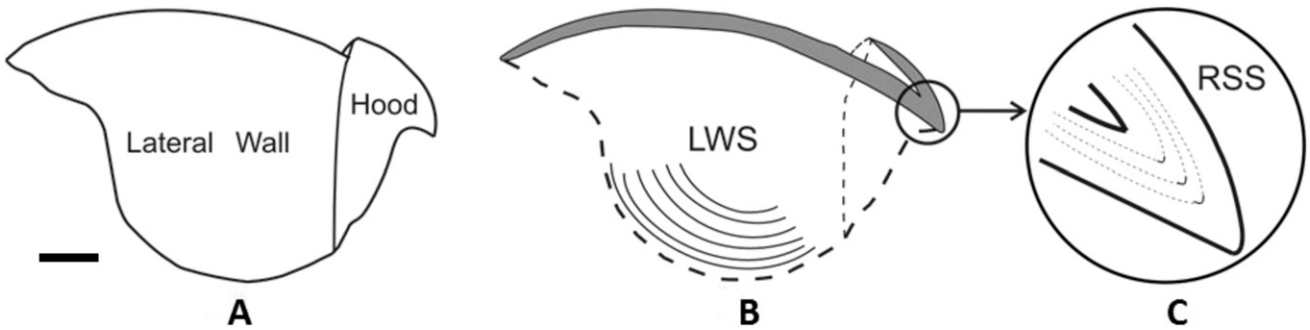

Figure 8. (A) Drawing of an adult cephalopod beak. Lateral view of the upper jaw in *Octopus vulgaris* (right = anterior, left = posterior). Bar ~2 mm. (B) Sagittal section showing the inner LWS bearing increments (lines). (C) Rostrum sagittal section (RSS) magnified and showing increments. From Perales-Raya et al. (2014b).

Liu et al. 2015, Fang et al. 2016, Hu et al. 2016), as well as age and event recording in the planktonic early stages of *O. vulgaris* (Franco-Santos et al. 2016, Garrido et al. 2016, Perales-Raya et al. 2017).

Beak analysis. Freezing the specimen is the preferred preservation method. After thawing and beak removal, they are cleaned and preserved in distilled water at cold temperature (~4°C). For longterm preservation, ethanol 70% is suitable, although it dehydrates the beak microstructure and visibility of rings could be affected. Before preparation, jaws are weighed and the main lengths are collected (Clarke 1986, Perales-Raya et al. 2010).

Several methodologies are used for beak preparation (see Figs. 8 and 9):

(1) Rostrum sagittal sections (RSS). The technique is based on the method developed by Perales-Raya and Hernández-González (1998) and improved by Perales-Raya et al. (2010). The rostrum region of either the upper or lower jaw is cut and embedded in resin (directly over a slide or in a small plastic mold), with the lateral side facing up. After hardening, the piece is ground with 1,200-grit waterproof sandpaper until reaching the central plane. Subsequently it is polished with 1 μm diamond paste. Aluminum oxide powder is also feasible for polishing, although diamond paste polishes faster to get a smooth surface ready for observation under the microscope. As the rostrum region is fully tanned, the increments are visible under reflected light (Fig. 9A) and it is not necessary to sand down both sides. Vertical episcopic (reflected) illumination is preferable to avoid interfering light reflections, as happens using oblique episcopic illumination. In squid samples, Liu et al. (2015) used a longer procedure: the sample was turned over, attached to the glass side, and ground again for observation under diascopic (transmitted) illumination. The best reading area is usually near the central axis of the RSS, where the hood and lateral wall joined, because increments are wider than other parts of the RSS. Caution should be taken when reading exactly along the central axis as branching into a couple of "false increments" has been reported in the squid *Dosidicus gigas* (d'Orbigny, 1835) (Liu et al. 2015). Increments located at the rostrum tip have undergone a process of erosion during the feeding process and it may bias increment count toward underestimation. Counting the first increments in the dorsal area of the RSS has been performed in several studies and species to prevent age underestimation (Perales-Raya et al. 2010, Liu et al. 2015). Magnification for RSS usually ranges between 150× and 300×.

(2) Lateral wall surfaces (LWS). The technique is based on the method developed by Clarke (1965) and improved by Hernández-López et al. (2001) and Perales-Raya et al. (2010). The upper jaw is sagittally sectioned with scissors to obtain two symmetrical half beaks, which are cleaned by hand with water. The inner surface of LWS is observed with some water to prevent dryness (Fig. 9B). Canali et al. (2011) immersed LWS briefly in 1M HCL and flattened them between two glass slides held together by adhesive tape. Vertical episcopic illumination is preferable, although either episcopic or diascopic illuminations are suitable in the untanned border of the LWS (Perales-Raya et al. 2010, 2014b). Magnification for LWS usually ranges between 50× and 150×. Some underestimation has been reported for LWS, although absolute increment count in LWS was closer to chronological age than that in RSS (Perales-Raya et al. 2014b). The latter is subject to more loss of material by erosion because it includes the most anterior region of the

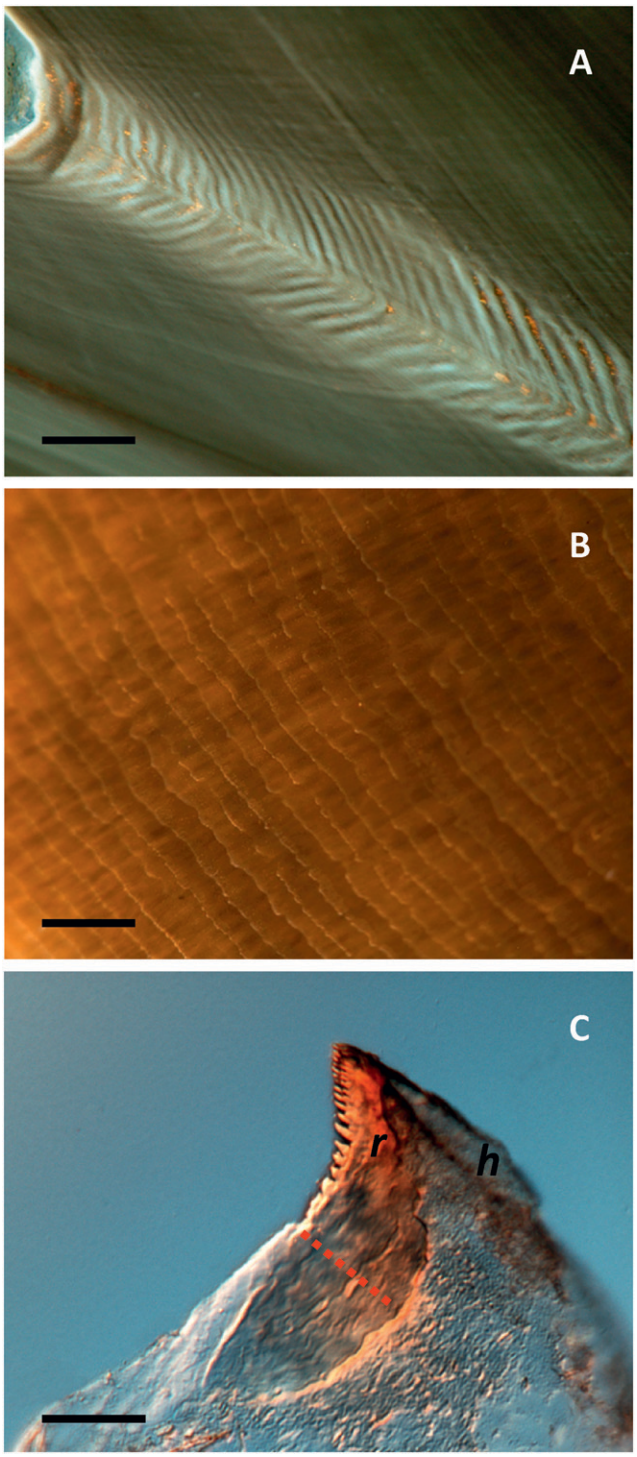

Figure 9. Growth increments in cephalopod beaks (*Octopus vulgaris*). (A) Increments in the RSS. Bar = 100 μm. (B) Increments in the inner LWS of the upper jaw. Bar = 200 μm. (C) Increments in the LRS of the upper jaw of paralarvae (*r* = rostrum; *h* = hood; dotted red line = width of the reading area). Bar = 500 μm.

rostrum tip, which is more exposed during predation on armored prey such as bivalves or crustaceans.

(3) A third technique is suitable for less pigmented beaks (e.g., early stages and species with adult translucent beaks) using the anterior pigmented region of the upper jaw (Fig. 9C), where daily increments have been observed under transmitted light (Perales-Raya et al. 2014b, Franco Santos et al. 2016). The reading area corresponds to the rostrum (Perales-Raya et al. 2017) where a sequence of thin increments is visible in the lateral rostrum surface (LRS). The upper jaw is sagittally sectioned with a scalpel to obtain two symmetrical half beaks under the binocular microscope and the inner surface of the LRS can be observed with some water. Differential interference contrast (DIC-Nomarski) is useful to improve the visibility of these increments under transmitted light. Minimum magnification for counting increments in LRS is usually 300×.

Daily increments in RSS, LWS, and LRS are counted directly under the microscope or using photos taken by the camera of an image analysis system. Identification of increments under the live camera mode is desirable for multifocal imagery when increments are unclear. Capturing images allows further analysis of the sample, for example, automatic measuring of increment widths and extrapolating distances in poorly defined areas. Age precision (*sensu* Campana 2001) is usually assessed by coefficient of variation for at least two independent readings.

LIPOFUSCIN

The measurement of lipofuscin is a pioneer approach in studying cephalopod age, particularly in taxa for which statoliths are not a reliable aging tool (as in cuttlefishes and octopods). Lipofuscin is an age pigment accumulated in tissues and its concentration was found not to be related to sex, temperature, or body weight in octopuses of the same age (Doubleday & Semmens 2011). The temperature factor could be important in lipofuscin accumulation rate, particularly among populations living at extremal temperatures compared with those living in their thermal comfort "mid-range." This method is expensive and not accurate enough for direct age estimations, so it could be more useful for relative aging such as splitting animals into age cohorts (Zielinski & Portner 2000, Doubleday & Semmens 2011, Gras et al. 2016).

EYE LENSES

Counting growth rings on eye lenses was another approach used for tentative aging of cephalopods with unreadable statoliths, like in octopus. Lenses were preserved in neutralized formalin. After decalcification and dehydration, the eye lens was impregnated with paraffin, and histological slides were made using a microtome and stained with hematoxylin–eosin. Application of this approach produced seemingly reasonable estimations of age in *Enteroctopus megalocyathus* (Gould, 1852) (Cárdenas et al. 2011). Later validation of this method in the octopus *Octopus maya* (Voss & Solis, 1966) raised in captivity demonstrated that eye lens increment counts did not correspond to the observed age, and if there is any periodicity, it is subdaily (Rodríguez-Domínguez et al. 2013).

SUMMARY

In summary, for many commercially important cephalopod species groups, a diverse range of age-registering structures and techniques exist for age and potentially growth rate estimation. With an increasing need to manage expanding fisheries, the use of techniques such as those described here is likely to become of paramount importance for many cephalopod stocks, if indeed they are not already used in this context. Several techniques (such as lipofuscin and eye lenses) are in their infancy and, with continued research, may greatly improve the management of key cephalopod species for which more traditional methods, such as statolith and beak reading, are not appropriate. Likewise, more traditional methods may also be improved with future developments in analytical techniques, enabling a greater amount of information to be extracted from each sample.

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